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PRINCIPAL INVESTIGATOR: Junxuan Lu, Ph.D.

CONTRACTING ORGANIZATION: AMC Cancer Research Center
Lakewood, Colorado 80214

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1. Introduction

During this current reporting period, the transfer of DOD grant fund from my past employment AMC Cancer Research Center, Lakewood, CO to my present employment Hormel Institute, University of Minnesota was not accomplished. Therefore, the execution of the aims was suspended awaiting for the funds. Here we offer an update of the progress report filed for the previous reporting period.

The purpose of this research is to gain a better understanding of the biochemical pathways and molecular targets for the selective induction of apoptosis signaling and execution of PCa cells by methyl Se/selenol. We hypothesized that methyl Se/selenol inhibits PI₃K-AKT survival pathway leading to the activation of caspase-dependent apoptosis execution in PCa cells. The specific aims are to delineate the caspase-mediated execution pathways of apoptosis (Objective 1) and to critically test the role of PI₃K-AKT survival pathway in apoptosis signaling (Objective 2) induced by methyl Se/selenol.

2. Key accomplishments

- 2.1 Induction of caspase-mediated apoptosis and cell cycle G₁ arrest by selenium metabolite methylselenol (*Molecular Carcinogenesis*, 2002).

Previous work based on mono-methyl selenium compounds that are putative precursors of methylselenol has strongly implicated this metabolite for inducing caspase-mediated apoptosis of human prostate carcinoma and leukemia cells and for inducing G₁ arrest in human vascular endothelial and cancer epithelial cells. To test the hypothesis that methylselenol itself was responsible for exerting these cellular effects, we examined the apoptotic action on DU145 human prostate cancer cells and G₁ arrest effect on human umbilical vein endothelial cells (HUVEC) of methylselenol generated with seleno-L-methionine as a substrate for L-methionine- α -deamino- γ -mercaptomethane lyase (EC4.4.1.11, also known as methioninase). Exposure of DU145 cells to methylselenol so generated in the sub-micromolar range led to caspase-mediated cleavage of poly(ADP-ribose)polymerase, nucleosomal DNA fragmentation and morphological apoptosis and resulted in a similar profile of biochemical effects as exemplified by the inhibition of phosphorylation of protein kinase B/AKT and extracellularly-regulated kinases 1/2 when compared to methylseleninic acid (MSeA) exposure. In HUVEC, methylselenol exposure recapitulated the G₁ arrest action of MSeA on mitogen-stimulated G₁ progression during mid- to late-G₁ and this stage-specificity was mimicked by inhibitors of the phosphatidylinositol 3-kinase. Taken together, the results support methylselenol as an active selenium metabolite for inducing caspase-mediated apoptosis and cell cycle G₁ arrest. This **cell-free methylselenol generation system** is expected to have a significant utility for studying the biochemical and molecular targeting mechanisms of this critical metabolite and may constitute the basis of a novel therapeutic approach for cancer.

- 2.2 Distinct effects of methylseleninic acid vs. selenite on apoptosis, cell cycle and protein kinase pathways in DU145 human prostate cancer cells (*Molecular Cancer Therapeutics*, 2002).

While the anti-cancer mechanisms have not been clearly defined, one hypothesis relates to selenium metabolites, especially the mono-methyl selenium pool, generated under supranutritional selenium supplementation. To explore potential molecular targets for mediating the chemopreventive activity, we contrasted the effects of methylseleninic acid (MSeA), a novel precursor of methylselenol, vs. sodium selenite, a representative of the hydrogen selenide metabolite pool, on apoptosis execution, cell cycle distribution and selected protein kinases in DU145 human prostate cancer cells. Exposure of DU145 cells to 3 μ M MSeA led to a profound G₁ arrest at 24 h and exposure to greater concentrations led to not only G₁ arrest, but also to DNA fragmentation and caspase-mediated cleavage of poly(ADP-ribose)polymerase (PARP), two biochemical hallmarks of apoptosis. Immunoblot analyses indicated that G₁ arrest induced by the sub-apoptogenic doses of MSeA was associated with increased expression of P27kip1 and P21cip1, but apoptosis was accompanied by dose-dependent decreases of phosphorylation of protein kinase AKT and extracellular signal regulated kinase (ERK1/2) in the absence of any phosphorylation change in p38 mitogen activated protein kinase (p38MAPK) and c-Jun N-terminal kinase (JNK1/2). In contrast, selenite exposure caused S phase arrest and caspase-independent apoptotic DNA fragmentation, which were associated with decreased expression of P27kip1 and P21cip1 and increased phosphorylation of AKT/PKB, JNK1/2 and p38MAPK. Whereas apoptosis induction by MSeA exposure was not sensitive to superoxide dismutase added into the cell culture medium, cell detachment and DNA nucleosomal fragmentation induced by selenite exposure were greatly attenuated by this enzyme, supporting a chemical mediator role of superoxide for these processes. In spite of a temporal relationship of AKT and ERK1/2 dephosphorylation changes before the onset of PARP cleavage in MSeA-exposed cells, experiments with phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 did not show an enhancing effect of specific blocking of AKT on MSeA-induction of PARP cleavage. Taken together, exposure of DU145 cells to MSeA vs. selenite (under ample serum culture condition, i.e., 10%) induced differential patterns of cell cycle arrest and apoptosis execution as well as distinct patterns of effects on AKT, ERK1/2, JNK1/2 and P38MAPK phosphorylation and p27kip1 and p21cip1 expression. Multiple molecular pathways are likely differentially targeted by selenium metabolite pools to mediate cancer chemoprevention.

- 2.3 Refractoriness of LNCaP prostate cancer cells to methyl selenium induction of caspase-mediated apoptosis (AACR Abstract 100956, 2003)

We have shown that methylselenol and its precursor compounds induce caspase-mediated apoptosis resembling detachment-induced apoptosis "anoikis" in DU145 prostate cancer cells and have observed that cell death is associated with decreased phosphorylation of protein kinase AKT involved in cell survival signaling. Because DU145 cells contain wild type PTEN tumor suppressor gene, which negatively regulates phosphatidyl inositol-3-phosphate kinase (PI3K)-AKT pathway rendering low basal AKT activity in these cells, we tested whether mutant PTEN-bearing LNCaP prostate cancer cells with higher basal AKT activity were refractory to methyl selenium/selenol induction of apoptosis under ample serum culture condition. In dose-response

experiments with 24 h exposure in the presence of 10% fetal bovine serum, LNCaP cells withstood much greater doses of methylseleninic acid as judged by cleavage of poly(ADP-ribose)polymerase and DNA fragmentation in comparison to DU145 cells. Similarly, methylselenol generated in the cell culture medium by methioninase using selenomethionine as a substrate failed to kill LNCaP cells at doses that efficiently killed DU145 cells. Nevertheless, LNCaP cells were more sensitive than DU145 cells to undergo apoptosis induced by sodium selenite exposure, which induces DNA single strand breaks. These results indicate that LNCaP cells are not cross-resistant to death signaling by selenium with a genotoxic mechanism(s). The role of PI3K-AKT in refractoriness to methyl Se exposure will be studied in coming years.

- 2.4 Selenite induces caspase-mediated apoptosis and p53 phosphorylation on serine-15 in LNCaP prostate cancer cells (AACR abstract 100969, 2003)

Our earlier work has shown that sodium selenite, a precursor of the hydrogen selenide metabolite pool induces DNA nucleosomal fragmentation and apoptosis of p53-mutant DU145 prostate cancer cells without caspase-mediated cleavage of poly(ADP-ribose)polymerase (PARP) (i.e., caspase-independent), whereas methylselenol and its precursors induce caspase-mediated apoptosis. Because selenite exposure is genotoxic through induction of DNA single strand breaks, and certain types of DNA damage induce p53-dependent apoptosis, we investigated whether selenite exposure of human LNCaP prostate cancer cells, which contain wild type functional p53 tumor suppressor gene, leads to caspase activation in a monolayer cell culture model. The results show that exposure of LNCaP cells for 24 h to lower micromolar concentrations of selenite led to dose-dependent DNA apoptotic fragmentation, procaspase activation and PARP cleavage. These changes were accompanied by p53 phosphorylation on Ser-15, but not on several other sites, and also by an induction of the expression of p53-target protein p21cip1 with no increase of total p53 content. A general caspase inhibitor zVADfmk and the specific inhibitors for caspase-8, 9 or 3 blocked PARP cleavage efficiently and decreased DNA fragmentation by a major extent. We are currently investigating whether the death signaling pathway from selenite exposure involves the following: selenite -> hydrogen selenide -> superoxide -> DNA single strand breaks -> p53 phosphorylation -> caspases.

3. Reportable outcomes

3.1 Peer reviewed Publications

Wang, Z. Jiang, C. and Lu JX. Induction of caspase-mediated apoptosis and cell cycle G₁ arrest by selenium metabolite methylselenol. *Mol Carcinogenesis*, 34: 113-120, 2002.

Jiang C, Wang Z, Ganther H, Lü J. Distinct effects of methylseleninic acid versus selenite on apoptosis, cell cycle, and protein kinase pathways in DU145 human prostate cancer cells. *Mol Cancer Ther*. 2002 Oct;1(12):1059-66.

3.2 Meeting abstracts

Junxuan Lu, Cheng Jiang. Refractoriness of LNCaP prostate cancer cells to methyl selenium induction of caspase-mediated apoptosis. Abstract No. 100956 American Association for Cancer Research 94th Annual meeting. Washington DC, 2003.

Cheng Jiang, Junxuan Lu. Selenite induces caspase-mediated apoptosis and p53 phosphorylation on serine-15 in Incap prostate cancer cell. Abstract No. 100969 American Association for Cancer Research 94th Annual meeting. Washington, DC, 2003.

J Lu, C. Jiang, Z. Wang, and H. Ganther. Selenium and cancer angioprevention: metabolite-specific anti-angiogenic attributes. Era of Hope 2002 DoD Breast Cancer Research Program Meeting September 25-28, 2002, at the Orange County Convention Center, Orlando, Florida. (Poster presentation).

4. Conclusions

Work conducted so far has further strengthened the differential pathways involved in signaling and executing apoptosis induced by different selenium metabolite pools. The specific role of PI3K-AKT pathway in methyl Se induced caspase-mediated death merits investigation under conditions of serum and oxygen deprivation conditions that exist prevalently inside growing lesions and cancers. Transfection of constitutive mutants into DU145 and LNCaP cells will be carried out in the next year to delineate their roles. The cell-free methylselenol system refined here will be useful to address efficacy of death induction in different cell lines without complication of metabolism. In addition, the role of p53 in mediating selenium-induced death should be investigated in reference to specific metabolite pools. The prevailing conclusion in the literature is that selenium induction of apoptosis is independent of p53 (1). That assertion merits further inquiry in light of our findings. As early lesions are more likely to retain p53 wild type function than full blown cancer cells, a p53-mediated caspase-dependent apoptosis induction by certain selenium metabolites may in part account for the observation of preferential sensitivity of early lesions to selenium intervention than cancer cells (2).

5. References

1. Kaeck M, Lu J, Strange R, Ip C, Ganther HE, Thompson HJ. Differential induction of growth arrest inducible genes by selenium compounds. *Biochem Pharmacol.* 1997 Apr 4;53(7):921-6.
2. Medina D, Shepherd FS. Selenium inhibition of the neoplastic transformation in preneoplastic mammary cell populations. *Cancer Lett.* 1984 Sep;24(2):227-34.